

### *Hypothesis*

# A possible biological role of the electron transfer between tyrosine and tryptophan

## Gating of ion channels

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Experiments have demonstrated that four tryptophan residues are located near the tetrodotoxin binding site in Na<sup>+</sup> channels, and that conserved tyrosine and tryptophan residues are located in the pore-forming region of voltage-sensitive K<sup>+</sup> channels. This paper proposes an activation mechanism involving electron transfer between these residues. The K<sup>+</sup> channel may be closed by four tyrosine residues forming hydrogen bonds with each other. After electron transfer, these hydrogen bonds will be broken, thereby opening the channel. The Na<sup>+</sup> channel could be activated by a similar mechanism. This idea can be tested directly by observing tyrosine or tryptophan radicals when the channels are in the open state.

Ion channel; Electron transfer; Tyrosine radical; Tryptophan radical

## 1. INTRODUCTION

"... it seems difficult to escape the conclusion that the changes in ionic permeability depend on the movement of some component of the membrane which behaves as though it had a large charge or dipole moment."

Hodgkin and Huxley, 1952 [1]

"The present paper shows that charge transfer between tryptophan and tyrosine units can be induced in a variety of proteins and proposes that it could be a fundamental process in the normal biological action."

Prutz and co-workers, 1982 [2]

Voltage-sensitive ion channels are a special class of proteins embedded in a variety of cell membranes [3]. The opening and closing of these channel proteins have been demonstrated to be associated with 'charge movement' [4,5], whose physical origin remains unclear. It could be the movement of charged residues [6], or the long-range electron transfer between two redox centers [7]. The primary structure of these channel proteins consists of four similar domains, each having six helical transmembrane segments, S1–S6 [6]. For K<sup>+</sup> channels, the linker between S5 and S6 has been identified [8–10] as the pore-forming region (referred to as H5 or SS1–SS2). The amino acid sequences in this region are quite conserved for various voltage-sensitive K<sup>+</sup> channels [11]. From the primary structure, the tyrosine at posi-

tion 445 and tryptophan at 434 or 435 look far apart (*Shaker* numbering is used throughout this paper). However, by studying the TEA (tetraethylammonium) blockade from either the intracellular or extracellular side, Yellen et al. [10] found that the secondary structure of the SS1–SS2 region is a  $\beta$ -strand, which makes a turn near the position 441. Therefore, in the tertiary structure the distance between tyrosine and tryptophan could be within the range for electron transfer between them [12].

Is the presence of tyrosine and tryptophan in the pore-forming region just a coincidence, or are they playing a major role in the channel's function? The electron transfer between tyrosine and tryptophan has been suggested to be a fundamental process in natural biological actions [2,12], but no example has been well documented yet. However, it has been induced in a variety of proteins by artificially using N<sub>3</sub> radicals to oxidize tryptophan [13]. The time scale of the electron transfer system ranges from 10<sup>-5</sup> to 10<sup>-2</sup> seconds [13]. The activation of voltage-sensitive ion channels falls into this range [1]. The rate of electron transfer increases with increasing temperature [13,14], which is also consistent with the temperature-dependence of the channel's activation [1,15]. More quantitatively, for the electron transfer between tyrosine and tryptophan in the protein,  $\beta$ -lactoglobulins, the rate increases by a factor of 1.8 for every 10° increment in temperature. This Q<sub>10</sub> value is about the same as the charge movement associated with the opening of Na<sup>+</sup> channels [15].

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In this paper we propose a simple mechanism for the activation of K<sup>+</sup> channels by electron transfer between tyrosine and tryptophan.

## 2. ACTIVATION MECHANISMS

Fig. 1 shows a secondary structure of the SS1–SS2 region for one subunit of the *Shaker* K<sup>+</sup> channel which contains four identical subunits. The residues with side chains pointing toward the pore are indicated. This structure was obtained by Busath and his group [16] using computational molecular modeling, adjusted for the best fit of experimental results. In this structure, the side chains of tyrosine-445 and tryptophan-435 are pointing toward the pore. The two residues constitute the narrowest region of the pore. Furthermore, the side chains of Y445s from four subunits can make hydrogen bonds with each other (Fig. 2a). The hole formed by the four tyrosine side chains is rather small, as estimated from the length of hydrogen bonds. The center-to-center distance between oxygen atoms in O–H...O is about 2.8 Å [17]. The distance between two diagonal O atoms is then equal to 4.0 Å. Subtracting the van der Waals radius (1.46 Å) for O atoms [18], the diameter of the hole is only 1.1 Å, which is smaller than the crystal diameter of the K<sup>+</sup> ion (2.7 Å). Therefore, the hydrogen-bonded structure could be the 'closed' state of the channel, since under physiological conditions the ion movement is unlikely to break four hydrogen bonds.

Let us denote TyrOH to be the tyrosine side chain. After an electron jumps out of TyrOH, we have



The pK<sub>a</sub> of TyrOH<sup>•</sup> is less than zero [12]. Thus, the deprotonation reaction (1b) will occur at all pH values. The tyrosine becomes a radical, which has an unpaired electron (denoted by a dot beside oxygen). From Fig. 2b we see that there is no hydrogen bonding between tyrosine radicals. The side chains of tyrosine radicals may bend toward the extracellular or intracellular side, making more room for the ions to pass through. Therefore, the formation of tyrosine radicals at position 445 may correspond to the 'open' state of the channel. We note that the hole formed by the tyrosine radicals is surrounded by a ring of oxygen atoms which have been suggested to form the narrow selectivity filter [19]. If this region is indeed the selectivity filter, the size of the hole could be at most 3.3 × 3.3 Å [19].

Similar to reaction (1), tryptophan (denoted as TrpH) may become radical Trp<sup>•</sup> after losing an electron. The ability of a molecule to lose an electron is measured by its redox potential which has been determined to be 0.94 V (vs. NHE at pH 7.0 and 25°C) for the tyrosine couple TyrO<sup>•</sup>/TyrOH, and 1.05 V for the tryptophan couple Trp<sup>•</sup>/TrpH [20]. These values are not large, which led

Butler et al. [2] to speculate that tyrosine and tryptophan radicals could be involved in natural biological processes. The electric field from charged residues, hydrogen bondings and covalent interactions could lower the activation barrier and the potential energy at the acceptor site to induce electron transfer from the donor. We note that the redox potential difference between the tyrosine and tryptophan couple is only 0.11 V. Therefore, under proper conditions, electrons may transfer, not only between homogeneous species (tyrosine-to-tyrosine or tryptophan-to-tryptophan), but between tyrosine and tryptophan as well. Furthermore, both tyrosine and tryptophan contain aromatic rings where electrons are quite delocalized. This feature makes the tyrosine/tryptophan system capable of conducting long-range electron transfer [12]. With these properties, the tyrosine/tryptophan system could be used for biological control and signal transduction.

In the pore-forming region of most voltage-sensitive K<sup>+</sup> channels, there are two tryptophans, W434 and W435, near the tyrosine-445. The opening of K<sup>+</sup> channels may be associated with the electron transfer from Y445 to either one of tryptophans, or to both tryptophans consecutively. For *Shal* K<sup>+</sup> channels, W435 (*Shaker* numbering) is replaced by a tyrosine residue [11]. The gating kinetics of *Shal* is not dramatically different from other K<sup>+</sup> channels [21]. This may be due to the fact that the redox potential difference between tyrosine and tryptophan is small. If W434 or W435 is mutated to any amino acid other than tyrosine, much more dramatic effects could be observed. Mutations of W434 and W435 into serine have been found to result in non-functional channels [8].

As mentioned earlier, the N<sub>3</sub> radical can induce electron transfer from tyrosine to tryptophan by oxidizing tryptophan [13]. The radical has electron-withdrawing capability, which effectively lowers the potential energy of the transferring electron at the site where the radical attacks. The electron-withdrawing groups (O, F, N, etc.) have been found to be important for the activity of K<sup>+</sup> channel openers (e.g. Cromakalim and Diazoxide [22]). In natural physiological processes, voltage-sensitive ion channels are not activated by drugs but by depolarizing voltages of the order of a few tens of millivolts. The depolarizing field may change the potential energy of the transferring electron [7,23], thereby changing the probability for the electron to be located at either the donor (e.g. Y445) or the acceptor (e.g. W434 or 435). As a rough estimate, let us assume that the vertical distance between the donor and acceptor is about 10 Å. Then a voltage of 50 mV across the membrane with a thickness of 50 Å can change the relative potential energy between the two redox sites by only 10 meV – if the potential energy change is due to the applied field alone. This value is too small to account for the steep voltage-dependence of ion conductance in a certain range of depolarization [1]. The poten-

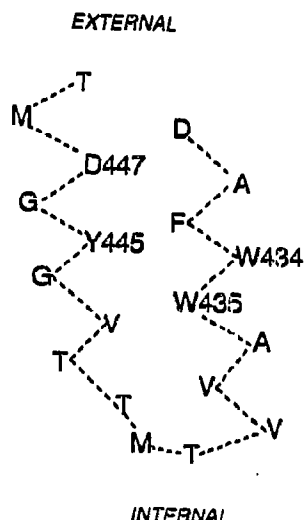


Fig. 1. Zig-zag representation for the secondary structure of the SS1-SS2 region of *Shaker* K<sup>+</sup> channel. The side chains of the residues on the inner edge are pointing toward the pore. Standard one-letter abbreviations for amino acids are used [10].

tial energy change should be at least 10-times larger. However, the applied field may change the distances between the charged residues (the 'voltage sensor') and the transferring electron, thereby changing the potential energy much more significantly. For instance, the interaction potential between two electronic charges is 2.06 eV for a separation of 7 Å, but increases to 2.4 eV for a separation of 6 Å in a vacuum environment.

The S4 segment contains several positively charged residues which have been postulated to be the voltage sensor [6]. Mutations of these charged residues affect gating kinetics, but in a non-systematic way, suggesting that other parts of the channel may also be involved in voltage sensitivity [24]. In Fig. 1, we see that a conserved residue, Asp-447 (D447), is located just above Y445. It seems that the depolarizing field (pointing outward) may pull the negatively charged group in D447 toward the side chain of Y445. The electron in Y445 will feel a stronger repulsive force and thus is more likely to jump to W434 or W435. The opening probability of voltage-sensitive K<sup>+</sup> channels has been found to decrease at lower external pH value [19], which may be due to the fact that, at lower pH, aspartate is more likely to be neutralized by protons, thereby unable to exert a repulsive field to induce electron transfer.

The possibility that aspartate may stimulate electron transfer to open the channel is interesting, considering that an aspartate derivative, *N*-methyl-D-aspartate (NMDA), is an important excitatory neurotransmitter. Upon binding of NMDA, its receptor opens to Ca<sup>2+</sup> ions. The opening probability can be modulated by oxidizing and reducing agents [25], suggesting that electron transfer might be involved. Excessive stimulation

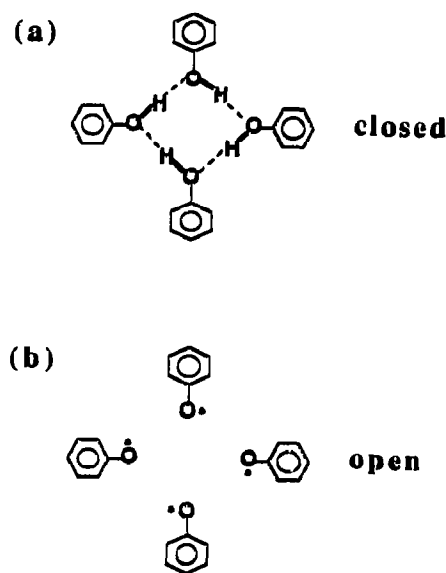


Fig. 2. Molecular structure of the tyrosine-445s from four subunits. (a) The hole formed by the hydrogen bonds between tyrosine side chains is too small for the ions to pass through. This could be the 'closed' state of the channel. (b) After electron transfer, the tyrosines become radicals which do not form hydrogen bonds with each other. This could be the 'open' state of the channel.

by NMDA can cause neuronal damage, which was found to be associated with a large amount of free radicals [26]. The exact binding site of NMDA is not known. However, it has been demonstrated that another neurotransmitter, acetylcholine, binds on a tyrosine residue in its nicotinic receptor [27]. The nicotinic acetylcholine receptor is also an ion channel, whose opening probability decreased dramatically when the specific tyrosine residue was mutated to other amino acids [27].

The activation mechanism presented here suggests that a K<sup>+</sup> channel may become fully open when four Y445s all become radicals due to four electron transfer processes. This mechanism is consistent with the Hodgkin-Huxley equations, where the potassium conductance is proportional to  $n^4$ , implying that four independent processes are needed to open one K<sup>+</sup> channel [1]. The molecular structure of Na<sup>+</sup> channels is less clear. However, if they are activated by a similar mechanism, the Hodgkin-Huxley equations for the sodium conductance, proportional to  $m^3$  [1], should imply that 'three' electron transfer processes are needed to open one Na<sup>+</sup> channel. This idea is consistent with an 'oxygen triad' model [28] for the activity of Batrachotoxin (BTX) and other alkaloid neurotoxins. These neurotoxins can open Na<sup>+</sup> channels at resting potentials [29]. By analyzing the structure-activity relationships for these alkaloid neurotoxins and their derivatives, Matsutani et al. [28] found that three oxygen atoms forming a triangle of a specific dimension are essential for the alkaloid neurotoxins to open the Na<sup>+</sup> channel. The

oxygen triad, similar to  $N_3$  radicals and the electron-withdrawing groups in  $K^+$  channel openers, may be able to attack acceptor sites, inducing three electron transfer processes from the donors. The tyrosines and/or tryptophans which could be interacting with the oxygen triad may not be exposed to the pore, since the size of BTX is much larger than a  $Na^+$  ion. It is unlikely that BTX can go into the pore without blocking the ion pathway. Because BTX and other alkaloid neurotoxins are quite hydrophobic, they may move into the protein interior to induce electron transfer, thereby opening the channel. Thus, the tyrosines and/or tryptophans which the oxygen triad may bind could be located in the pore-forming region with side chains pointing out from the pore. They could also be located in other regions. However, if the  $Na^+$  channel is activated by a mechanism similar to  $K^+$  channels, the tyrosines and/or tryptophans which donate electrons to the acceptor sites (where the oxygen triad binds) should be located in the pore-forming region.

From the homology between the primary structures of  $Na^+$  and  $K^+$  channels, we expect that the pore-forming region of  $Na^+$  channels is also located at the linker between S5 and S6 segments. This view is now strongly supported [30]. It has been suggested that tetrodotoxin (TTX) binds at the narrow selectivity filter of  $Na^+$  channels [31]. In rat  $Na^+$  channel II, the residues which may bind to TTX have been identified [30], as listed below:

Repeat I:	D384	F385	W386	E387
Repeat II:	E942	W943	I944	E945
Repeat III:	K1422	G1423	W1424	M1425
Repeat IV:	A1714	G1715	W1716	D1717

Mutations of the residues in the two columns at D384 and E387 affected the sensitivity of the channel to TTX binding. Incidentally, four tryptophan residues, W386, W943, W1424 and W1716, appear near the TTX binding site! Why are there so many tryptophans in the region which could be the narrow selectivity filter? Are they playing a role similar to the four Y445s in  $K^+$  channels? The detailed molecular structure and activation mechanism remain to be investigated.

It is possible that some of the four tryptophan residues may form hydrogen bonds with nearby residues to close the channel. After electron transfer from these tryptophans the hydrogen bonds will be broken, thereby opening the channel. The negatively charged residues in the E387 column, similar to D447 in  $K^+$  channels, may have some contribution in stimulating electron transfer upon depolarization. However, the  $Na^+$  channel contains much more charged residues in the pore-forming region than the  $K^+$  channel. The contribution from the E387 column may not be dominant. Mutation of E387 to glutamine still resulted in a functional channel, but the stage of the I-V curve was changed [32].

### 3. FUTURE TEST

To further test the model, we may use site-directed mutagenesis to see if the effects are as expected. For example, mutation of Y445 in  $K^+$  channels to any other amino acid should result in non-functional channels. For  $Na^+$  channels, if three of the four tryptophans near the TTX binding site are involved in the channel's gating, mutation of any of them should have significant effects on the channel's function. Mutation to tyrosine may have the least effect since its redox property is similar to tryptophan. On the other hand, we may also try to observe tyrosine or tryptophan radicals when the channels are in the open state. The tyrosine and tryptophan radicals have been observed in a few biological systems using electron paramagnetic resonance [33,34]. They may also be detected from absorption spectra: the tyrosine radical absorbs at 405 nm and tryptophan radical at 510 nm. This characteristic absorption has been used to demonstrate directly the electron transfer between tyrosine and tryptophan in a variety of proteins [13].

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